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14. ABSTRACT

The aim of this proposal is to isolate and characterize prostatic epithelial stem cells. This will be done as stem cell and tumor cell biology may be closely linked and stem cells may have a role in the etiology of cancer. Our results show that the prostate contains a small population of cells (<1%) capable of effluxing the Hoechst dye by an active process(the side population, SP), indicating that cells with features of stem cells are found in the prostate. As 96% of the SP cells express Sca-1, a protein expressed by stem cells of other origins, we isolated Sca-1 expressing cells and assayed their proliferative ability in an invivo prostate reconstitution assay. These results show that prostate regenerating ability resides in the Sca-1 expressing population. In addition, we show that prostate stem cells reside in the proximal region of ducts within the population that expresses high levels ofSca-1. We also show that bone marrow cells can differentiate into prostatic epithelial(basal) cells and fibroblasts with significantly more fibroblasts (7%) being of bone marrow origin than basal epithelial cells (1%).

Prostate stem cells, fluorescent activated cell sorting, Sca-1 antigen, bone marrow stem cells

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Introduction

The aim of this proposal is to isolate and characterize prostatic epithelial stem cells. This will be done as stem cell and tumor cell biology may be closely linked and stem cells are increasingly thought to have a role in the etiology of cancer (1-7). Stem cells and tumor cells have many common features including self-renewal, multi-drug resistance, telomerase expression and, in the instance of the prostate, androgen independence. Recently primitive normal prostate cells have been shown to express the Sca-1 antigen (8, 9). The progenitor cells are sensitive to oncogenic transformation (9) and Pten deletion leads to the expansion of a prostate progenitor cell population (10) indicating that progenitor prostate cells may be targets of transformation.

Stem cells from a variety of tissues have been shown to actively efflux the Hoechst dye (11) through the activity of multi-drug-resistance (MDR)-like proteins (12) and this property has been used to characterize and isolate them as a side population (SP).

There is also a compelling body of evidence that indicates that adult bone marrow (BM)-derived stem cells are able to differentiate into numerous types of tissues (13). Recent evidence also indicates that gastric cancer can originate from BM-derived cells (14). This data indicates that BM gives rise not only to normal differentiated cells in a variety of organs but that BM cells are also capable of being transformed within an organ and form tumors.

The aim of this proposal is to determine if cells with properties expressed by side population cells are located in the prostatic tissue of mice, to examine these cells for the expression of marker proteins and to determine if they have growth properties of stem cells. In addition we will determine if BM cells are able to 'home' to prostate and differentiate into normal tissues

Body

Tasks 2 and 3. To identify and isolate the side population (SP) of Hoechst-effluxing cells from the dorsal prostate (DP) and to identify the proteins expressed in SP cells.

Our previous progress report defined the conditions for labeling prostate cells with the Hoechst 33342 dye to identify and isolate the Hoechst-excluding prostate SP. We found that ~0.65% of dorsal prostate (DP) cells belong to the Hoechst-excluding SP and that 96% of SP cells expressed Sca-1 with 88% of cells expressing both Sca-1 and α6 integrin, two proteins found on the surface of a number of types of stem cells. We also showed that SP cells have a greater in vitro proliferative capacity than non-SP cells and formed both larger and more ducts in collagen gels than non-SP cells.

We were unable to do the in vivo experiments proposed in Task 3 due to the low numbers of SP cells obtained in the prostate (<1%) and due to their poor viability as they have an in vitro cloning efficiency of ~0.5%. This resulted in so few cells with the ability to proliferate that we were unable to obtain sufficient numbers of cells to insert under the renal capsule of recipient animals or to further fractionate and isolate them based on the expression of Sca-1 and α6 integrin. The Hoechst dye 33342 has been found to be toxic to some cells and can result in low viability after FACS sorting. As this was our experience we were unable to proceed with this approach. We will also be unable to do the in vivo intraprostatic inoculations of GFP-tagged FACS isolated SP cells proposed in Task 4. The low numbers of SP cells with growth capacity means that sufficient GFP-tagged SP cells will not be able to be obtained for these intraprostatic in vivo studies.

As we had shown that almost all the SP cells expressed Sca-1 and as SP cells have been shown to have stem cell properties in other systems we proceeded to isolate the Sca-1 expressing population from the proximal and remaining regions of ducts and compare their properties in in vivo assays. We found that Sca-1 is highly expressed in the proximal region of prostatic ducts and that prostatic stem cells can be isolated based on high levels of expression of Sca-1.

We found that 52 ± 11% of proximal cells express Sca-1 compared with 18 ± 7% of cells in the remaining regions of ducts. The proximal region also had far more cells (10 ± 1%) expressing high levels of Sca-1 (Sca-1^{high}) than remaining ductal regions (2 ± 1%) (8). We also found that the proximal region is considerably enriched (28 ± 4%) in Sca-1 expressing cells that co-express α6 integrin and Bcl-2 - two other antigens shown to be expressed by stem cells – than remaining regions (1 ± 1%) (8). These results show that there are striking differences in the distribution of cells expressing Sca-1, α6 integrin and Bcl-2 in different ductal regions. Cells with high levels of Sca-1 are predominantly confined to the proximal region and triple labeled cells with high levels of Sca-1 are almost exclusively confined to this region (8).

Sca-1 expressing cells have high in vivo regenerative potential

The ability to regenerate tissue in vivo is a characteristic of stem cells and this property has been used to identify various antigens, including Sca-1, as stem cell markers (15, 16). We therefore determined the growth potential of Sca-1 expressing cells isolated (using magnetic beads and antibodies to Sca-1) from the proximal and the remaining ductal regions and compared their proliferative potential in vivo with cells that did not express this antigen. These populations were combined with cells isolated from the urogenital sinus mesenchyme (UGM) of 18 day old embryos (inductive mesenchyme for prostatic tissue), inserted under the renal capsule of recipient male animals and the amount of prostatic tissue generated was measured after 8 weeks. Sca-1 expressing cells isolated from the proximal region formed significantly more prostatic tissue (17.1 fold; $p < 0.001$) than was obtained from the Sca-1 depleted proximal population (Fig. 1) (8).

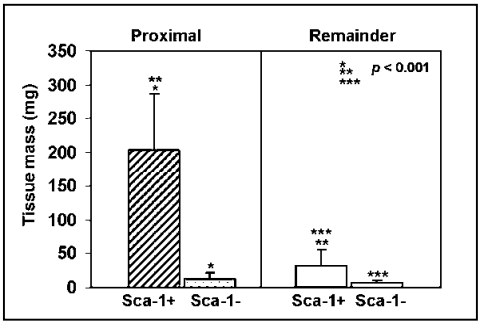

Fig. 1. Sca-1+ cells have greater in vivo regenerative capacity than Sca-1- cells. The growth of Sca-1+ and Sca-1- cells (10^5 cells) that were isolated from either the proximal or the remaining regions and transplanted under the RC was measured after 8 weeks. Sca-1+ cells, obtained from the proximal region formed 17.1 fold more prostatic tissue than Sca-1- cells ($p < 0.001$). Sca-1+ cells, obtained from the remaining ductal regions, had far less growth potential than Sca-1+ proximal cells ($p < 0.001$). Sca-1- cells from the remaining regions showed less growth than Sca-1+ cells from this region ($p < 0.001$).

Sca-1 expressing cells isolated from the remaining ductal regions also formed prostatic tissue under the RC (~32 mg) but formed far less tissue than observed for Sca-1 expressing cells isolated from the proximal region (~200 mg), indicating that these Sca-1 expressing populations differ markedly in their in vivo growth potential ($p < 0.001$). Sca-1 depleted cells isolated from the remaining regions of ducts formed very little sub-RC tissue.

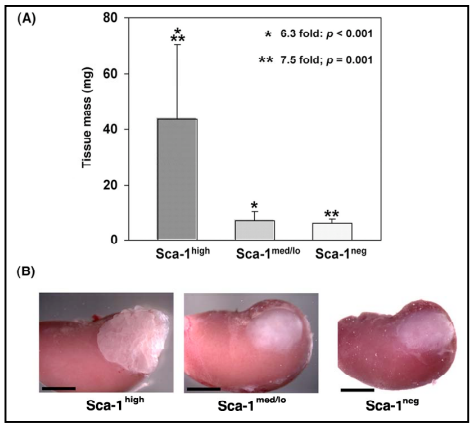

Fig. 2. Sca-1^{high} cells have greater in vivo regenerative capacity than cells that express lower levels of Sca-1. Cells were isolated from the proximal region and FACS sorted into Sca-1^{high}, Sca-1$^{med/lo}$ and Sca-1^{negative} fractions. The cell populations (3 x 10^4) were transplanted under the RC and the growth of prostatic tissue was measured after 10 weeks.
(A) Sca-1^{high} cells formed 6.3 fold more prostatic tissue than Sca-1$^{med/lo}$ cells ($p < 0.001$) and 7.5 fold more prostatic tissue than Sca-1^{negative} cells ($p = 0.001$).
(B) Prostate tissue initiated with 3 x 10^4 Sca-1^{high}, Sca-1 $^{med/lo}$ or Sca-1^{negative} cells isolated by FACS from the proximal region of ducts. Bars = 3 mm.

As our FACS data showed that cells expressing high levels of Sca-1 were confined predominantly to the proximal region of ducts, we determined whether Sca-1^{high} cells had greater growth potential than cells with medium/low Sca-1 expression. Proximal cell populations were FACS sorted into fractions containing cells with high median fluorescence intensity (MFI) (Sca-1^{high}), medium to low MFI (Sca-1$^{med/lo}$) and no Sca-1 expression (Sca-1^{negative}) and inserted under the RC of recipient animals. Sca-1^{high} cells formed significantly more prostatic tissue than Sca-1$^{med/lo}$ or Sca-1^{negative} cells (Fig. 2) (8).

Although the tissue growth obtained was less than in experiments using magnetic beads (due to the stress that the cells undergo during FACS sorting; note also that far fewer FACS sorted cells (3 x 10^4) were used), these results show that almost all of the in vivo regenerative potential is confined to cells that express high levels of Sca-1. The prostatic tissue obtained from Sca-1^{high} cells had normal prostatic histology comprising basal and luminal cells lining prostatic ducts. The lumina of the ducts contained abundant amounts of secretory material. In contrast, the tissue arising from Sca-1$^{med/lo}$ and Sca-1^{negative} cells contained more stroma with less of an epithelial component and little secretory material was noted within the ducts.

These results show that cells expressing Sca-1 have considerably more growth potential than those lacking this antigen and that the proliferative ability within the Sca-1 expressing proximal cells resides in cells that express high levels of this antigen. They also show that Sca-1 expressing cells residing in the proximal region are more primitive than those Sca-1 expressing cells in the remaining ductal regions as they have far higher regenerative capacity. These data indicate that stem cells reside within the Sca-1 expressing population in the proximal region, whereas cells with more limited growth potential (transit-amplifying cells), reside within the Sca-1 expressing cells in the remaining ductal regions.

Task 5. To determine if bone marrow-derived cells can differentiate into and 'home' to organs of the urogenital tract.

As transplanting mice with GFP-expressing donor bone marrow and subsequent cycling of them by adding and removing androgens is a prolonged experimental procedure we started Task 5. The aim of these experiments is to determine if bone marrow stem cells can migrate to the prostate and differentiate into prostatic epithelial or stromal cells. There is a considerable recent literature indicating that adult-derived bone marrow stem cells maintain extensive plasticity and differentiate into multiple

organs in the mouse (13, 17-19). In addition, gastric cancer has been shown to originate from bone marrow-derived cells (14) indicating that bone marrow-derived cells may also be relevant to the process of prostatic carcinogenesis.

GFP-expressing bone marrow cells were obtained from GFP-expressing C57Bl6 mice (Jackson labs, ME) and transplanted into irradiated recipient male mice. Engraftment was ascertained by analyzing peripheral blood of recipient animals for evidence of GFP-expressing cells. The recipient animals were castrated to induce prostatic involution. After 14 days testosterone pellets (Innovative Research, FL) were implanted subcutaneously to regenerate the prostate as we expected circulating bone marrow stem cells to engraft more readily into regenerating prostate tissue. We find very few bone marrow-derived GFP-expressing cells in the epithelial compartment of the prostate (0.9 ± 0.3%) and these are located only in the basal and not the luminal layer. In contrast, significant numbers of GFP-expressing cells that co-express vimentin are present in prostatic stroma (7.3 ± 1.7%). Preliminary analysis indicates that ~25% of vimentin positive cells (fibroblasts) in the stroma express GFP (stroma consists predominantly of fibroblasts and smooth muscle cells) indicating that many prostatic stromal cells originate from bone marrow. As vimentin is present in fibroblasts and not bone marrow cells this indicates that the GFP-expressing bone marrow cells have differentiated into prostatic stromal cells.

We plan to examine mice that have been androgen cycled for more prolonged periods of time and that have had bone marrow stem cells mobilized with G-CSF for evidence of prostatic engraftment. G-CSF increases the numbers of GFP-expressing bone marrow stem cells in the circulation. This should enhance GFP-stem cell incorporation into regenerating prostatic tissue.

As the Hoechst dye was toxic for the prostatic stem cell population we would like to measure the levels of aldehyde dehydrogenase in proximal cells. Stem cells from other origins have been shown to express high levels of aldehyde dehydrogenase (ALDH) activity which can be used for their functional isolation (20-22). As we have shown that stem cells express high levels of Sca-1 we propose measuring aldehyde dehydrogenase levels in conjunction with staining for Sca-1 to determine if cells expressing high levels of Sca-1 also have high levels of aldehyde dehydrogenase.

Key research accomplishments

1) We show that the proximal region of murine prostatic ducts is considerably enriched in cells that express high levels of the cell surface antigen, Sca-1.
2) Sca-1-expressing cells were isolated from the proximal region and remaining regions of ducts and assayed for proliferative potential in an in vivo prostate reconstitution assay. This showed that proximal Sca-1 expressing cells had far greater growth potential than Sca-1 expressing cells isolated from remaining regions of ducts which are likely to be a post-stem cell (transit-amplifying) compartment.
3) Almost all of the proliferative activity resided in the cells expressing high levels of Sca-1 indicating that prostatic stem cells reside within the Sca-1^{high} compartment.
4) Bone marrow can differentiate into both prostatic epithelium (basal cells) and stroma. However, significantly more stromal cells are of bone marrow origin than epithelial cells.

Reportable outcomes

1) Publication - see attached paper:
P.E. Burger, X Xiong, S Coetzee, S.N. Salm, D. Moscatelli, K Goto, E. L. Wilson. Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with a high capacity to reconstitute prostatic tissue. Proc Natl Acad Sci USA 102; 7180-7185 (2005).

2) The PI presented a talk incorporating data from this proposal at the NIH sponsored Cellular Niches Workshop, May 16-17, 2005, Bethesda, MD
The properties and regulation of prostatic stem cells and their relevance to prostatic diseases.
E Lynette Wilson, Ken Goto, Sarah Salm, Patricia Burger, Sandra Coetzee, Xiaozhong Xiong, Ellen Shapiro, Herbert Lepor and David Moscatelli.

3) The following posters incorporating data from this proposal were presented at the Cellular Niches Workshop, May 16-17, 2005.
Sca-1 expression identifies a progenitor/stem cell population in the proximal region of murine prostatic ducts with a high capacity to reconstitute prostatic tissue
Patricia E. Burger, Sandra Coetzee, Xiaozhong Xiong , Sarah N. Salm, David Moscatelli, Ken Goto and E. Lynette Wilson.

Bone Marrow Cells are able to generate prostatic epithelial and stromal cells.
Xiaozhong Xiong, Ken Goto, Yasuhiro Ebihara, Sarah Salm, Sandra Coetzee, Christopher Ontiveros, David Moscatelli, Makio Ogawa and E Lynette Wilson.

3) A grant was submitted to NIH on 1 November 2005 incorporating data obtained from this proposal.

Conclusions

We have shown that the prostate contains a small population of cells (~0.6%) that exclude the Hoechst dye and whose ability to exclude the dye is inhibited by verapamil. Almost all cells (96%) of this side population (SP) express the antigen Sca-1 and most of them (88%) co-express Sca-1 with ∝6 integrin. As the Hoechst dye was toxic to the small SP, sufficient numbers of cells could not be obtained for in vivo prostate reconstitution experiments to determine their stem cell origin. As most SP cells expressed Sca-1, we isolated cells based on Sca-1 expression and showed that the stem cell population in the prostate resides within that fraction of cells expressing high levels of this antigen. We also show that cells eressing high levels of Sca-1 express ∝6 integrin and Bcl-2, an anti-apoptotic protein found in tumor cells and stem cells.

We have therefore, for the first time, provided a profile of prostatic stem cells based on protein expression that enables their purification and assay. This population can now be compared with prostate tumor cell populations to determine their similarity or differences. As tumor stem cell populations have recently been isolated we expect that normal stem cells and tumor stem cells will have overlapping features. The identification of prostate stem cells may enable development of new therapies which may ablate both normal and tumor stem cells. As the prostate is not an essential organ the ablation of normal prostatic stem cells in conjunction with carcinoma cells will not adversely affect the health of the patient.

Our data also indicate that bone marrow stem cells can differentiate into both epithelial and stromal compartments of the prostate with significantly more prostatic stromal cells than epithelial cells being of bone marrow origin. This finding may have considerable implications for the etiology of prostatic diseases as gastric cancer has been shown originate from bone marrow-derived cells (14).

Personnel supported by this grant

E Lynette Wilson Effort 15%
Rashmi Gupta Effort 100%

References

1. Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001;414(6859):105-11.
2. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ, Clarke MF. Prospective identification of tumorigenic breast cancer cells. Proc Natl Acad Sci U S A 2003;100(7):3983-8.
3. Al-Hajj M, Clarke MF. Self-renewal and solid tumor stem cells. Oncogene 2004;23(43):7274-82.
4. Pardal R, Clarke MF, Morrison SJ. Applying the principles of stem-cell biology to cancer. Nat Rev Cancer 2003;3(12):895-902.
5. Singh SK, Hawkins C, Clarke ID, Squire JA, Bayani J, Hide T, Henkelman RM, Cusimano MD, Dirks PB. Identification of human brain tumour initiating cells. Nature 2004;432(7015):396-401.
6. Huff CA, Matsui W, Smith BD, Jones RJ. The paradox of response and survival in cancer therapeutics. Blood 2006;107(2):431-4.
7. Li Y, Welm B, Podsypanina K, Huang S, Chamorro M, Zhang X, Rowlands T, Egeblad M, Cowin P, Werb Z, Tan LK, Rosen JM, Varmus HE. Evidence that transgenes encoding components of the Wnt signaling pathway preferentially induce mammary cancers from progenitor cells. Proc Natl Acad Sci U S A 2003;100(26):15853-8.
8. Burger PE, Xiong X, Coetzee S, Salm SN, Moscatelli D, Goto K, Wilson EL. Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue. Proc Natl Acad Sci U S A 2005;102(20):7180-5.
9. Xin L, Lawson DA, Witte ON. The Sca-1 cell surface marker enriches for a prostate-regenerating cell subpopulation that can initiate prostate tumorigenesis. Proc Natl Acad Sci U S A 2005;102(19):6942-7.
10. Wang S, Garcia AJ, Wu M, Lawson DA, Witte ON, Wu H. Pten deletion leads to the expansion of a prostatic stem/progenitor cell subpopulation and tumor initiation. Proc Natl Acad Sci U S A 2006;103(5):1480-5.
11. Goodell MA. Multipotential stem cells and 'side population' cells. Cytotherapy 2002;4(6):507-8.
12. Zhou S, Schuetz JD, Bunting KD, Colapietro AM, Sampath J, Morris JJ, Lagutina I, Grosveld GC, Osawa M, Nakauchi H, Sorrentino BP. The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. Nat Med 2001;7(9):1028-34.
13. Krause DS, Theise ND, Collector MI, Henegariu O, Hwang S, Gardner R, Neutzel S, Sharkis SJ. Multi-organ, multi-lineage engraftment by a single bone marrow-derived stem cell. Cell 2001;105(3):369-77.

14. Houghton J, Stoicov C, Nomura S, Rogers AB, Carlson J, Li H, Cai X, Fox JG, Goldenring JR, Wang TC. Gastric cancer originating from bone marrow-derived cells. Science 2004;306(5701):1568-71.
15. Spangrude GJ, Heimfeld S, Weissman IL. Purification and characterization of mouse hematopoietic stem cells. Science 1988;241(4861):58-62.
16. Welm BE, Tepera SB, Venezia T, Graubert TA, Rosen JM, Goodell MA. Sca-1(pos) cells in the mouse mammary gland represent an enriched progenitor cell population. Dev Biol 2002;245(1):42-56.
17. Petersen BE, Bowen WC, Patrene KD, Mars WM, Sullivan AK, Murase N, Boggs SS, Greenberger JS, Goff JP. Bone marrow as a potential source of hepatic oval cells. Science 1999;284(5417):1168-70.
18. Ferrari G, Cusella-De Angelis G, Coletta M, Paolucci E, Stornaiuolo A, Cossu G, Mavilio F. Muscle regeneration by bone marrow-derived myogenic progenitors. Science 1998;279(5356):1528-30.
19. Ebihara Y, Masuya M, Larue AC, Fleming PA, Visconti RP, Minamiguchi H, Drake CJ, Ogawa M. Hematopoietic origins of fibroblasts: II. In vitro studies of fibroblasts, CFU-F, and fibrocytes. Exp Hematol 2006;34(2):219-29.
20. Hess DA, Meyerrose TE, Wirthlin L, Craft TP, Herrbrich PE, Creer MH, Nolta JA. Functional characterization of highly purified human hematopoietic repopulating cells isolated according to aldehyde dehydrogenase activity. Blood 2004;104(6):1648-55.
21. Armstrong L, Stojkovic M, Dimmick I, Ahmad S, Stojkovic P, Hole N, Lako M. Phenotypic characterization of murine primitive hematopoietic progenitor cells isolated on basis of aldehyde dehydrogenase activity. Stem Cells 2004;22(7):1142-51.
22. Corti S, Locatelli F, Papadimitriou D, Donadoni C, Salani S, Del Bo R, Strazzer S, Bresolin N, Comi GP. Identification of a primitive brain-derived neural stem cell population based on aldehyde dehydrogenase activity. Stem Cells 2005.

Appendices

See attached publication

Sca-1 expression identifies stem cells in the proximal region of prostatic ducts with high capacity to reconstitute prostatic tissue

Patricia E. Burger*[†‡], Xiaozhong Xiong[†§], Sandra Coetzee[†§], Sarah N. Salm[§¶], David Moscatelli[§‖], Ken Goto[§**], and E. Lynette Wilson*[§‖††]

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Communicated by David D. Sabatini, New York University School of Medicine, New York, NY, April 6, 2005 (received for review February 25, 2005)

We previously showed that prostatic stem cells are concentrated in the proximal regions of prostatic ducts. We now report that these stem cells can be purified from isolated proximal duct regions by virtue of their high expression of the cell surface protein stem cell antigen 1 (Sca-1). In an *in vivo* prostate reconstitution assay, the purified Sca-1-expressing cell population isolated from the proximal region of ducts was more effective in generating prostatic tissue than a comparable population of Sca-1-depleted cells (203.0 ± 83.1 mg vs. 11.9 ± 9.2 mg) or a population of Sca-1-expressing cells isolated from the remaining regions of ducts (transit-amplifying cells) (31.9 ± 24.1 mg). Almost all of the proliferative capacity of the proximal duct Sca-1-expressing cell population resides within the fraction of cells that express high levels of Sca-1 (top one-third), with the proximal region of prostatic ducts containing 7.2-fold more Sca-1^{high} cells than the remaining regions. More than 60% of the high-expressing cells coexpress α6 integrin and the antiapoptotic factor Bcl-2, markers that are also characteristic of stem cells of other origins. Further stratification of the phenotype of the stem cells may enable the development of rational therapies for treating prostate cancer and benign prostatic hyperplasia.

prostate | α6 integrin | Bcl-2

Stem cell biology and tumorigenesis may be closely linked, and stem cells may have a role in the etiology of cancer (1–5). Stem cells and tumor cells have many common features, including self-renewal, multidrug resistance, telomerase expression, and, in the case of the prostate, androgen independence. Prostatic stem cells do not require androgens for survival, as evidenced by normal prostatic regeneration after >30 cycles of androgen ablation and supplementation, which results in involution and normal regeneration of this gland (6). Because prostatic carcinoma usually progresses to an androgen-independent tumor (which may reflect a stem cell-like phenotype), an understanding of prostate stem cell biology is important for devising preventative or therapeutic approaches for prostate cancer. In addition to being a source of carcinomas, stem cells may also give rise to benign prostatic hyperplasia (7). The isolation and characterization of these stem cells is likely to increase our understanding of normal prostate physiology, and it may also lead to new therapeutic approaches for two of the most common diseases afflicting men.

The murine prostate consists of a branched ductal network with each duct consisting of proximal (adjacent to the urethra), intermediate, and distal regions. Actively proliferating cells (transit-amplifying cells) are located in the distal region of the ducts (8), whereas cells with stem cell features are concentrated in the proximal ductal region (9). Thus, cells in the proximal region are quiescent and have high proliferative potential, and isolated single cells from this region can give rise to branched glandular ductal structures *in vitro* (9). In addition, cell suspensions derived from the proximal region form significantly more prostatic tissue in an *in vivo* transplantation model than those obtained from other prostatic regions. Furthermore, cells obtained from this transplanted tissue are again able to give rise to prostatic tissue when reinoculated into other animals (unpublished data), confirming the presence in the proximal region of stem cells with regenerative capacity.

Because stem cells in other organs have been identified by their expression of specific antigens, such as a cell surface protein known as stem cell antigen 1 (Sca-1), α6 integrin, and Bcl-2, we determined whether these antigens could be used to identify the stem cell population in the proximal region of ducts. Sca-1 is expressed by stem/progenitor cells from a variety of tissues, such as hematopoietic tissue (10), cardiac tissue (11), mammary gland (12), skin (13), muscle (14), and testis (15). α6 integrin (CD49f) is expressed by primitive cells in the liver (16) and skin (17), and anti-α6 integrin antibodies have been used to enrich for spermatogonial stem cells from mouse testis (18). Bcl-2, an antiapoptotic protein (19), may protect primitive cells from death and is expressed by hematopoietic, keratinocyte, and colon stem cells (20–22).

We have identified a candidate population of prostatic stem cells in the proximal region of murine prostatic ducts that expresses high levels of Sca-1, in conjunction with α6 integrin and Bcl-2. Cells with these properties are almost absent from the remaining regions of ducts. We show that Sca-1-expressing cells isolated from the proximal region regenerate abundant normal functional prostatic ducts in an *in vivo* transplantation assay, whereas cells that do not express this antigen form very little tissue. These results establish that prostatic stem cells reside within the Sca-1-expressing population in the proximal region of ducts and provide a means of isolating the stem cells for further characterization.

Materials and Methods

Animals. C57BL/6 mice, athymic nude mice, and CDIGS rats were housed in the animal research facilities of the University of Cape Town or New York University, and all experiments were performed in compliance with institutional review board requirements.

Antibodies and Control Immunoglobulins (IgGs). Fluorescein isothiocyanate (FITC)-conjugated rat anti-mouse Sca-1, rat anti-mouse

Abbreviations: MFI, mean fluorescence intensity; PE, phycoerythrin; Sca-1, stem cell antigen 1; UGM, urogenital sinus mesenchyme.

[†]P.E.B., X.X., and S.C. contributed equally to this work.

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Table 1. Expression of Sca-1, α6 integrin, and Bcl-2 by cells from the proximal region of prostatic ducts compared with cells from the remaining ductal regions

Phenotype	No. of experiments	Proximal expression, %	Remainder expression, %	Increase, fold	*P*
Sca-1$^+$	16	51.8 ± 10.5	17.7 ± 7.2	2.9	<0.0001
α6 integrin$^+$	13	40.8 ± 10.0	21.1 ± 11.4	1.9	<0.0001
Bcl-2$^+$	12	42.1 ± 7.0	27.5 ± 8.2	1.5	<0.0001
Sca-1$^+$ α6 integrin$^+$ Bcl-2$^+$	3	27.5 ± 4.4	1.4 ± 0.8	19.6	<0.01
Sca-1^{high}*	12	15.9 ± 5.2	2.2 ± 1.4	7.2	<0.00001
Sca-1^{high}* α6 integrin$^+$ Bcl-2$^+$	3	9.8 ± 1.2	0.1 ± 0.06	98.0	<0.01

*Sca-1+ cells with fluorescence intensities in the upper one-third were defined as Sca-1^{high} cells.

α6 integrin (CD49f) FITC, and rat IgG 2a FITC were obtained from BD Biosciences, Bedford, MA. Phycoerythrin (PE)-conjugated rat anti-mouse Sca-1, rat IgG-2a PE, rat IgG, mouse anti-mouse CD16/32, rat anti-mouse Sca-1 biotin, rat IgG2a biotin, and streptavidin-conjugated allophycocyanin (APC) were from Caltag Laboratories, Burlingame, CA. Mouse anti-Bcl-2 PE was purchased from Santa Cruz Biotechnology, and IgG1 PE was obtained from DAKO.

Cell Preparation and FACS Analysis. The dorsal, ventral, and lateral prostates were removed from C57BL/6 mice (6 weeks old) and dissected into two regions: (*i*) the proximal region, which includes those ducts nearest the urethra, and (*ii*) the remaining region, which includes the intermediate and distal ducts. Cell digests (9) were resuspended in FACS buffer [PBS containing BSA (0.1%), sodium azide (0.01%), and aprotinin (20 μg/ml)]. Fc receptors were blocked with mouse CD16/32 antibodies and rat IgG, and the cells were incubated with antibody or control IgG for 30 min on ice and washed with FACS buffer. In some experiments, the dye 7-aminoactinomycin D (1 μg/ml) was added 5 min before analysis, so that dead cells could be excluded. Bcl-2 expression was determined in paraformaldehyde-fixed cells, permeabilized with Tween 20 (Merck-Schuchardt, Hohenbrunn, Germany). Antibodies to Sca-1, conjugated to PE, FITC, or biotin, were used in conjunction with antibodies to α6 integrin conjugated to FITC or antibodies to Bcl-2 conjugated to PE, to determine the incidence of coexpression of Sca-1 and these antigens. Cells were analyzed on a FACSCalibur flow cytometer (Becton Dickinson), using CELLQUEST software (Becton Dickinson). Sca-1$^+$ cells with fluorescent intensities in the upper one-third were defined as Sca-1^{high} cells.

Implantation of Grafts Under the Renal Capsule. Cells (1×10^5 or 3×10^4) from different regions of prostatic ducts were combined

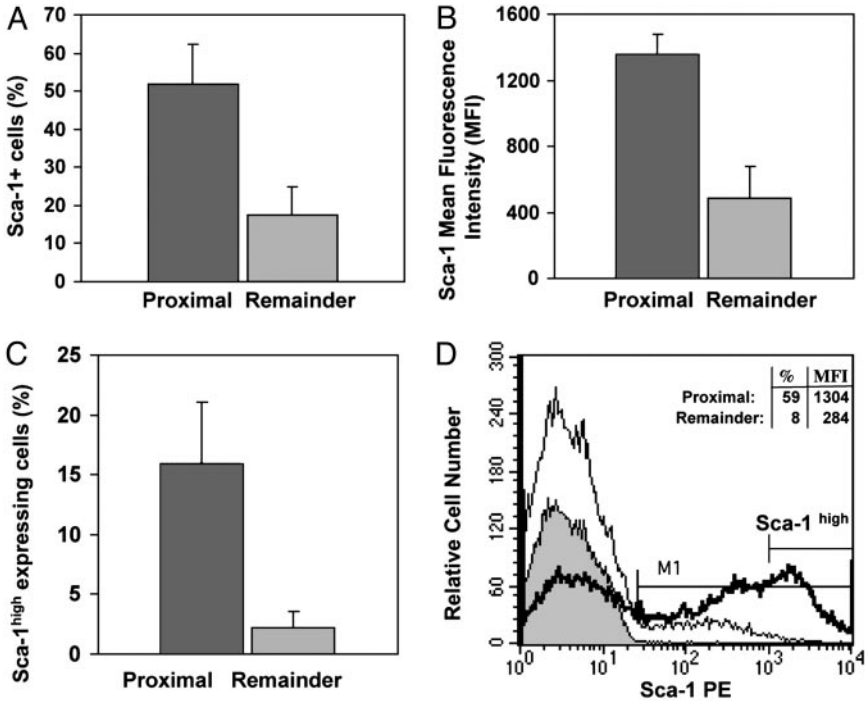

Fig. 1. Sca-1 is highly expressed by cells in the proximal region of prostatic ducts. The expression of Sca-1 by cell digests from the proximal and remaining regions of prostatic ducts was determined. (*A*) The proximal region contained 2.9-fold more Sca-1-expressing cells than the remaining ductal regions ($P < 0.0001$). (*B*) Cells from the proximal region expressed 2.8-fold more molecules of Sca-1 per cell (higher MFI) than cells from the remaining regions ($P < 0.01$). (*C*) Cells with high levels of Sca-1 expression (cells with fluorescence intensities in the upper one-third) were 7.2-fold more prevalent in the proximal region than in the remaining regions ($P < 0.00001$). Results in *A–C* are means of at least three experiments. (*D*) A representative histogram (from one of five experiments) of Sca-1 expression by viable (7-aminoactinomycin D-negative) cells from the proximal region (thick line) and the remaining regions (thin line) of ducts shows the difference in Sca-1 expression between these two regions. The gray-filled histogram represents the appropriate IgG control. The marker M1 is placed so that <1% of control cells are positive. A second marker denotes Sca-1^{high} cells.

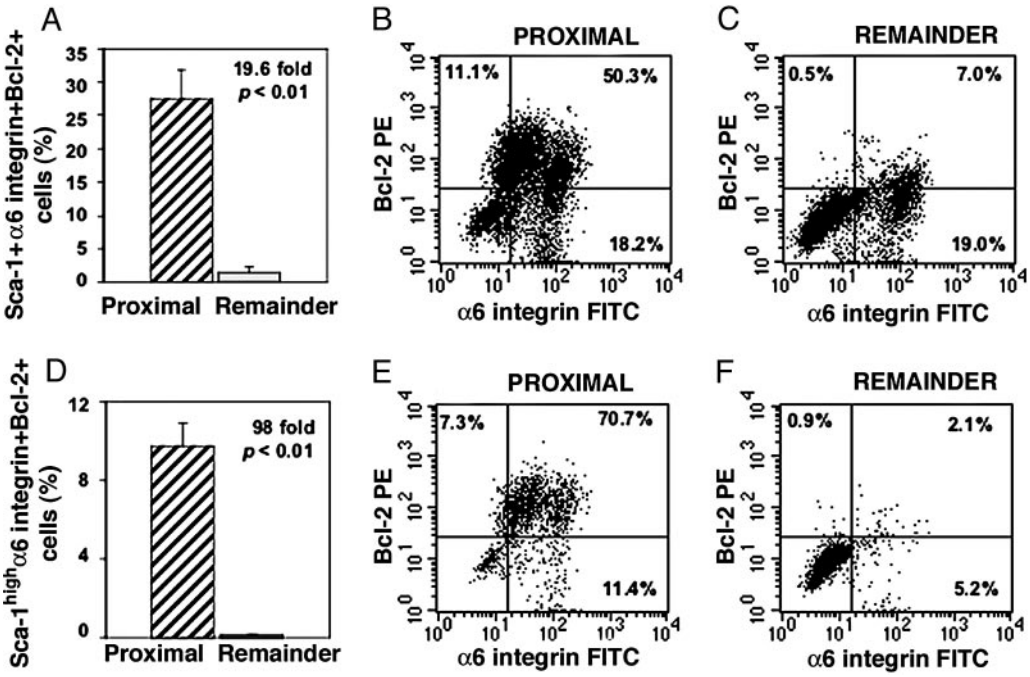

Fig. 2. The proximal region is considerably enriched in Sca-1-expressing cells that coexpress α6 integrin and Bcl-2. Three-color FACS analysis was performed to determine the incidence of Sca-1$^+$ α6 integrin$^+$ Bcl-2$^+$ cells (*A–C*) and Sca-1^{high} α6 integrin$^+$ Bcl-2$^+$ cells (*D–F*) in the proximal and remaining regions of ducts. (*A*) The proximal region contained 19.6-fold more Sca-1$^+$ α6 integrin$^+$ Bcl-2$^+$ cells than the remaining regions ($P < 0.01$). (*B* and *C*) In these representative dot plots, 50.3% of proximal Sca-1$^+$ cells coexpressed both α6 integrin and Bcl-2 (*B*), whereas 7.0% of cells from the remaining regions coexpressed these antigens (*C*). (*D*) Analysis of triple-labeled cells expressing high levels of Sca-1 showed that the proximal region contained 98-fold more Sca-1^{high} α6 integrin$^+$ Bcl-2$^+$ cells than the remaining regions ($P < 0.01$). (*E* and *F*) For these dot plots, 70% of proximal Sca-1^{high} cells coexpressed both α6 integrin and Bcl-2 (*E*), whereas 2% of cells from the remaining regions were Sca-1^{high} α6 integrin$^+$ Bcl-2$^+$ (*F*). The results are the mean of three experiments.

with urogenital sinus mesenchyme (UGM) cells (2.5×10^5) and resuspended in 30 μl of type 1 collagen (BD Biosciences). The collagen grafts were inserted under the renal capsule (23). Each experiment contained grafts of UGM alone to ensure that tissue growth did not result from contaminating urogenital sinus epithelial cells. Grafts were harvested and weighed after 8–10 weeks. UGM was isolated from the urogenital sinus of embryos (18 days old) from CDIGS rats (23–25).

Isolation of Sca-1-Expressing Cells. Prostatic duct digests were enriched for Sca-1-expressing cells by immunomagnetic separation, using magnetically activated cell sorter microbeads coated with antibodies to Sca-1 and the MiniMACS magnetically activated cell sorter system (Miltenyi Biotec, Auburn, CA). In some experiments, cells were sorted by FACS into various fractions (Sca-1^{high}, Sca-1$^{med/lo}$ or Sca-1^{neg}) according to the mean fluorescence intensity (MFI) of Sca-1 expression by the cells.

Statistical Analysis. The results are depicted as the means and standard deviations of each set of data. Comparisons between groups were made by using the two-tailed, paired Student *t* test, or in the case of different sized samples, the Mann–Whitney *U* test. A *P* value of <0.05 is considered statistically significant.

Results

Cells in the Proximal Region of Murine Prostatic Ducts Coexpress High Levels of Sca-1, α6 Integrin, and Bcl-2. We have shown that cells with stem cell features (quiescence and high proliferative potential) are concentrated in the proximal region of prostatic ducts (9). By using FACS analysis, we determined whether the expression of three antigens, Sca-1, α6 integrin, and Bcl-2, known to be expressed by stem cells of other origins (10–18, 20–22), differs between the proximal and remaining ductal regions.

We found that these three antigens are expressed by at least some cells in all regions of the ducts, but significant differences were noted in their distribution. They were expressed by a substantially higher proportion of cells in the proximal region than in the remaining regions (Table 1), and the levels of expression of each antigen (MFI) were higher in proximal cells than in cells of the remaining ductal regions. The proximal region contained a 2.9-fold ($P < 0.0001$) higher proportion of Sca-1-expressing cells that had a 2.8-fold-higher MFI ($P < 0.01$) than cells from the remaining ductal regions (Fig. 1 *A* and *B* and Table 1). Because high levels of Sca-1 are found on purified populations of other types of stem cells (14, 15, 26, 27), we determined the location of cells with high MFI for Sca-1. The proximal region of ducts contained 7.2-fold more cells with high levels of Sca-1 (Sca-1$^+$ cells with fluorescence intensities in the upper one-third) than the remaining regions (Table 1; $P < 0.00001$; Fig. 1 *C* and *D*), indicating that Sca-1^{high} cells are concentrated proximally.

Determination of the coexpression of all three antigens indicated that cells from the proximal region contain significantly more (19.6-fold; $P < 0.01$) Sca-1$^+$ α6 integrin$^+$ Bcl-2$^+$ cells (27.5 ± 4.4%) than those from the remaining regions (1.4 ± 0.8%) (Fig. 2 *A–C* and Table 1). Analysis of the proximal region for cells expressing high levels of Sca-1 together with α6 integrin and Bcl-2 (Sca-1^{high} α6 integrin$^+$ Bcl-2$^+$ cells) revealed that 98-fold more triple-labeled cells reside in the proximal compared with the other regions of ducts (9.8 ± 1.2% vs. 0.1 ± 0.06%, $P < 0.01$; Fig. 2 *D–F* and Table 1). In addition, each antigen alone was expressed by more cells (Table 1) and with a higher MFI (data not shown) in the proximal region compared with remaining regions.

These results show that there are striking differences in the distribution of cells expressing Sca-1, α6 integrin, and Bcl-2 in

different ductal regions. Cells with high levels of Sca-1 are predominantly confined to the proximal region and triple-labeled cells with high levels of Sca-1 are almost exclusively confined to this region.

Sca-1-Expressing Cells Have High *in Vivo* Proliferative Potential. The ability to regenerate tissue *in vivo* is a characteristic of stem cells, and this property has been used to identify various antigens, including Sca-1, as stem cell markers. For example, Sca-1-expressing cells isolated from bone marrow are able to reconstitute all blood cell types (10), and mammary epithelial cells enriched for Sca-1 can reconstitute the mammary gland *in vivo* and have greater growth potential than Sca-1-depleted cells (12).

We therefore determined the growth potential of Sca-1-expressing cells isolated from the proximal and the remaining ductal regions and compared their proliferative potential *in vivo* with cells that did not express this antigen. Sca-1-expressing (Sca-1^+) and Sca-1-depleted (Sca-1^-) populations were isolated from digests of the proximal and the remaining ductal regions by using antibodies to Sca-1 and magnetic microbeads. These populations were combined with cells isolated from the UGM [inductive mesenchyme for prostatic tissue (23–25)] and inserted under the renal capsule of recipient male animals, and the amount of prostatic tissue generated was measured after 8 weeks. Sca-1^+ cells isolated from the proximal region formed significantly more prostatic tissue (203.0 ± 83.1 mg; 17.1-fold) than was obtained from the Sca-1^- proximal population (11.9 ± 9.2 mg; $P < 0.001$) (Fig. 3 *A* and *B*). Sca-1^+ cells isolated from the remaining ductal regions also formed prostatic tissue under the renal capsule (31.9 ± 24.1 mg) but formed far less tissue than observed for Sca-1^+ cells isolated from the proximal region (203.0 ± 83.1 mg), indicating that these two Sca-1^+ populations differ markedly in their *in vivo* growth potential ($P < 0.001$). Sca-1^- cells isolated from the remaining regions of ducts formed very little subrenal capsule tissue (6.6 ± 5.0 mg).

Because our FACS data showed that cells expressing high levels of Sca-1 were confined predominantly to the proximal region of ducts, we determined whether Sca-1^{high} cells had greater growth potential than cells with medium/low Sca-1 expression. Proximal cell populations were FACS-sorted into fractions containing cells with high MFI (Sca-1^{high}), medium to low MFI (Sca-$1^{med/lo}$) and no Sca-1 expression (Sca-1^{neg}) and inserted under the renal capsule of recipient animals. Sca-1^{high} cells formed significantly more prostatic tissue (43.7 ± 26.8 mg; 6.3-fold) than Sca-$1^{med/lo}$ cells (6.9 ± 3.6 mg; $P < 0.001$) and 7.5-fold more tissue than Sca-1^{neg} cells (5.8 ± 1.6 mg; $P = 0.001$) (Fig. 4 *A–C*). Although the tissue growth obtained was less than in experiments using magnetic beads (because of the stress that the cells undergo during FACS sorting), these results show that almost all of the *in vivo* growth potential is confined to cells that express high levels of Sca-1. The prostatic tissue obtained from Sca-1^{high} cells had normal prostatic histology comprising basal and luminal cells lining prostatic ducts. The lumens of the ducts contained abundant amounts of secretory material (Fig. 4*C*). In contrast, the tissue arising from Sca-$1^{med/lo}$ and Sca-1^{neg} cells contained more stroma with less of an epithelial component, and little secretory material was generally noted within the ducts (Fig. 4*C*).

These results show that cells expressing Sca-1 have considerably more growth potential than those lacking this antigen, and that the proliferative ability within the Sca-1-expressing proximal cells resides in cells that express high levels of this antigen. They also show that Sca-1-expressing cells residing in the proximal region are more primitive than those Sca-1-expressing cells in the remaining ductal regions, as they have far higher proliferative capacity. These data indicate that stem cells reside within the Sca-1-expressing population in the proximal region, whereas the transit-amplifying cells, with more limited growth potential, reside within the Sca-1-expressing cells in the remaining ductal regions.

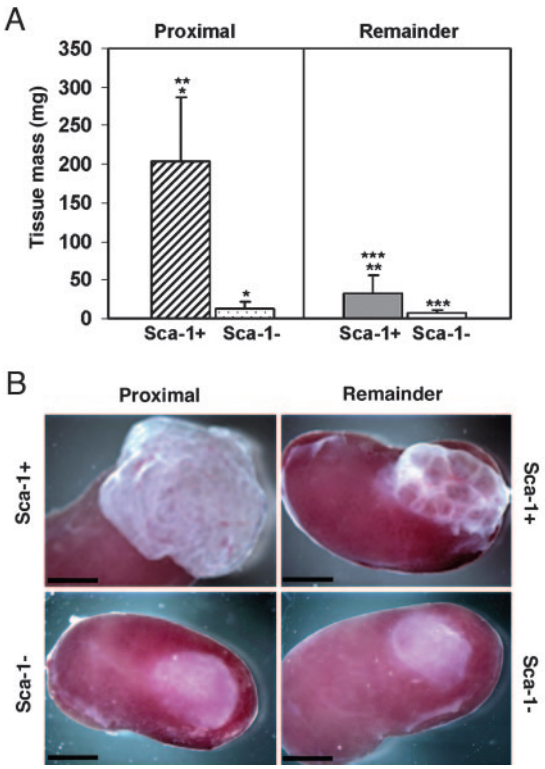

Fig. 3. Sca-1^+ cells have greater *in vivo* proliferative capacity than Sca-1^- cells. (*A*) The growth of Sca-1^+ and Sca-1^- cells (10^5 cells) that were isolated from either the proximal region or the remaining regions of ducts and transplanted under the renal capsule was measured after 8 weeks. Sca-1^+ cells obtained from the proximal region formed 17.1-fold more prostatic tissue than Sca-1^- cells (*, $P < 0.001$). Sca-1^+ cells obtained from the remaining ductal regions had far less growth potential than Sca-1^+ proximal cells (**, $P < 0.001$). Sca-1^- cells from the remaining regions showed less growth than Sca-1^+ cells from this region (***, $P < 0.001$). The results are the means of two experiments, using the data obtained from the inoculation of a total of 7, 11, 14, and 12 kidneys with Sca-1^+ and Sca-1^- cells from the proximal and remaining regions, respectively. (*B*) Prostate tissue under the renal capsule initiated with 10^5 Sca-1^+ or Sca-1^- cells from either the proximal region or the remaining regions. (Scale bars: 3 mm.)

Discussion

We show that Sca-1-expressing cells, isolated from the proximal region of ducts, form significantly more prostatic tissue *in vivo* than cells that lack this antigen and that most of the proliferative ability resides in cells that express high levels of this antigen. In addition, Sca-1-expressing cells isolated from the proximal region have far greater proliferative potential than Sca-1-expressing cells isolated from the remaining ductal regions (which express lower levels of Sca-1 per cell than proximal cells). The Sca-1^{high} cells that coexpress α6 integrin and Bcl-2 are almost exclusively confined to the proximal region of ducts, indicating that prostate stem cells express high levels of Sca-1 together with α6 integrin and Bcl-2.

Sca-1 is expressed by stem cells from a variety of origins including hematopoietic tissue, heart, mammary gland, skin, muscle, and testis (10–15). Although a ligand for Sca-1 has not been identified, Sca-1 appears to be important for the self-renewal of mesenchymal (28) and hematopoietic (29) stem cells. Sca-$1^{-/-}$ mice have greatly reduced bone mass resulting from a primary defect in the self-renewal capacity of early mesenchymal progenitor cells (28). In addition, hematopoietic stem cells from Sca-$1^{-/-}$ mice have a

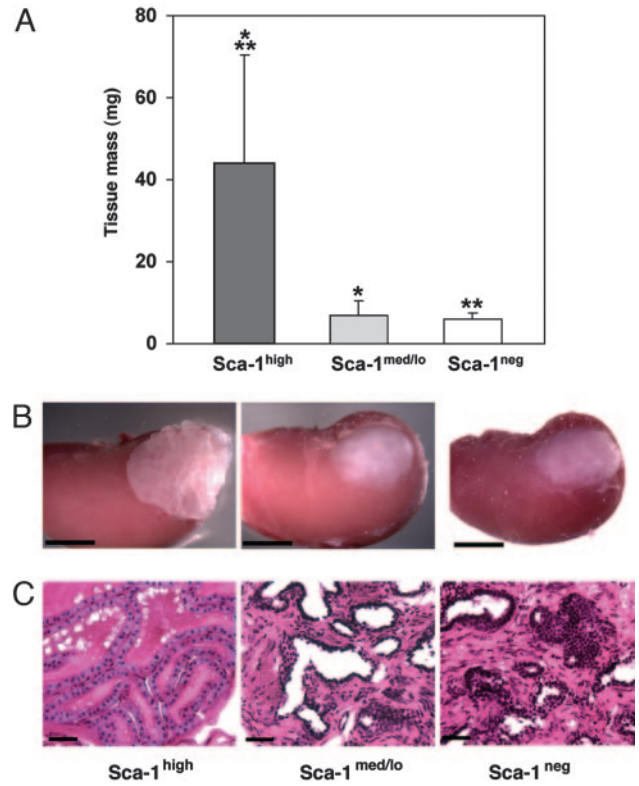

Fig. 4. Sca-1^{high} cells have greater *in vivo* proliferative capacity than cells that express lower levels of Sca-1. Cells were isolated from the proximal region and sorted by FACS into Sca-1^{high}, Sca-1$^{med/lo}$ and Sca-1^{neg} fractions according to the level of Sca-1 expression. The cell populations (3×10^4) were transplanted under the renal capsule and the growth of prostatic tissue was measured after 10 weeks. (*A*) Sca-1^{high} cells formed 6.3-fold more prostatic tissue than Sca-1$^{med/lo}$ cells (*, $P <$ 0.001) and 7.5-fold more prostatic tissue than Sca-1^{neg} cells (**, $P = 0.001$). These results are the means of two experiments, using the data obtained from the inoculation of a total of 10, 9, and 10 kidneys with cell populations containing Sca-1^{high}, Sca-1$^{med/lo}$ and Sca-1^{neg} cells, respectively. (*B*) Prostate tissue initiated with 3×10^4 Sca-1^{high}, Sca-1$^{med/lo}$, or Sca-1^{neg} cells isolated by FACS from the proximal region of ducts. (Scale bars: 3 mm.) (*C*) Paraffin sections stained with hematoxylin and eosin showing the morphology of prostatic tissue arising from Sca-1^{high}, Sca-1$^{med/lo}$ or Sca-1^{neg} cells. The prostatic tissue obtained from Sca-1^{high} cells had normal prostatic histology comprising basal and luminal cells lining prostatic ducts. The lumens of the ducts were filled with secretory material. The tissue arising from Sca-1$^{med/lo}$ and Sca-1^{neg} cells contained increased stroma with less of an epithelial component, and little secretory material was noted within the ducts. (Scale bars: 40 μm.)

decreased repopulation potential and manifest a lower engraftment of secondary transplants than cells from wild-type mice (29), indicating that Sca-1 is required for self-renewal. These findings are consistent with our data showing that Sca-1^{neg} cells have little capacity to generate prostatic tissue when implanted under the renal capsule, and indicate that Sca-1 may also be involved in the self-renewal of stem cells in the prostate.

Stem cells are rare cells, and, because large numbers of cells isolated from prostatic ducts express Sca-1, it is unlikely that all Sca-1-expressing cells are stem cells. Our data, in fact, indicate that prostatic stem cells reside in the Sca-1^{high} population that also expresses α6 integrin and Bcl-2. The presence of α6 integrin together with high levels of Sca-1 is also characteristic of spermatogonial stem cells (15). Stem cells from other origins also express α6 integrin. The gene for this integrin was the only common gene identified in a study using transcriptional profiling to identify genes expressed by stem cells of embryonic, neural, hematopoietic, and retinal origin (30). Keratinocyte stem cells also express high levels of α6 integrin (31), and these cells have enhanced long-term proliferative potential (32).

Members of the integrin family are important regulators of stem cell function (33). Keratinocyte and putative prostate stem cells are more adhesive than the more mature transit-amplifying cells, and putative human prostate stem cells express high levels of α2 integrin (34–36). It is possible that a number of members of the integrin family are expressed by stem cells because there is recent evidence that the adhesive properties of integrins may be involved in maintaining stem cells within their niche (37, 38). Because stem cells and cancer cells have many similar properties (1–5), it is of interest that changes in the expression of integrins, particularly $\alpha 6\beta 4$ integrin, are implicated in tumorigenesis and invasion and that the α6 integrins play a role in the progression of cancer (39–41).

The prostate cells from the proximal region that express high levels of Sca-1 also coexpress the antigen Bcl-2. The presence of Bcl-2 in Sca-1-expressing prostate stem cells may protect these cells from apoptotic death. Stem cells are needed for the lifetime of their host, and mechanisms to protect them from death are important to ensure their long-term survival. The Bcl-2 protein suppresses apoptosis (19) and is present in many long-lived cells (42). Bcl-2 protects hematopoietic and keratinocyte stem cells from apoptotic death (21, 43), and over-expression of Bcl-2 increases the numbers of hematopoietic stem cells *in vivo* (20) and protects hematopoietic stem cells from the harmful effects of a number of chemotherapeutic agents, thus ensuring their survival (44). The expression of Bcl-2 by the prostate stem cell population that has high levels of Sca-1 and significant *in vivo* proliferative potential is therefore likely to ensure the long-term survival of this cell population.

High levels of Bcl-2 in the proximal stem cell region may also be required to protect these cells from apoptosis that accompanies androgen withdrawal. Castration results in an increase in TGF-β levels (45), leading to apoptosis and involution of the more distal regions of the gland, whereas the proximal region is relatively unchanged (46, 47). We find a TGF-β signaling gradient in prostatic ducts, with high levels of signaling in the quiescent proximal region (high Bcl-2 expression) and low levels of signaling in the distal region (low Bcl-2 expression) (S.N.S., P.E.B., S.C., K.G., D.M., and E.L.W., unpublished data). The proximal region is therefore protected from TGF-β-mediated apoptosis by high Bcl-2 expression. Aberrant regulation of Bcl-2 expression may contribute to the etiology of prostatic diseases such as benign prostatic hyperplasia (48), proliferative inflammatory atrophy, which is a regenerative lesion that may give rise to prostate cancer (49), and prostate cancer itself (50). In addition, the over-expression of Bcl-2 is implicated in the formation of hormone-independent prostate tumors because it inhibits the apoptotic effect of TGF-β and androgens (51). The identification of the phenotype of prostatic stem cells that express high levels of Bcl-2 may therefore aid in identifying the target cells from which these lesions originate.

The identification of other antigens expressed by the population of cells that express Sca-1, α6 integrin, and Bcl-2 may result in the definition of a more comprehensive phenotype for prostate stem cells. For example, the expression of antigens such as CD133 (prominin), which has been found on human putative prostatic stem cells (52), signaling molecules, such as Wnt, Notch, and Hedgehog, that are involved in stem cell renewal and maintaining stem cell niches (53, 54), and members of the Polycomb family, such as Bmi1 and EZH2 (55, 56), may further stratify the prostatic stem cell phenotype. Because cancers may arise from mutations in stem cells (2, 4, 5) and because benign prostatic hyperplasia may result from aberrant proliferation of these cells (7), the identification of the stem cell phenotype of prostate cells may permit the development of rational targeted therapies for treating both conditions.

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1. Al-Hajj, M., Wicha, M. S., Benito-Hernandez, A., Morrison, S. J. & Clarke, M. F. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 3983–3988.
2. Al-Hajj, M. & Clarke, M. F. (2004) *Oncogene* **23,** 7274–7282.
3. Lapidot, T., Sirard, C., Vormoor, J., Murdoch, B., Hoang, T., Caceres-Cortes, J., Minden, M., Paterson, B., Caligiuri, M. A. & Dick, J. E. (1994) *Nature* **367,** 645–648.
4. Pardal, R., Clarke, M. F. & Morrison, S. J. (2003) *Nat. Rev. Cancer* **3,** 895–902.
5. Reya, T., Morrison, S. J., Clarke, M. F. & Weissman, I. L. (2001) *Nature* **414,** 105–111.
6. Isaacs, J. T. (1985) in *Benign Prostatic Hyperplasia*, eds. Rodgers, C. H., Coffey, D. S., Cunha, G., Grayshack, J. T., Henman, R., Jr., & Horton, R. (Natl. Inst. of Health, Washington, DC), DHHS Publ. No. 87-2881, pp. 85–94.
7. De Marzo, A. M., Nelson, W. G., Meeker, A. K. & Coffey, D. S. (1998) *J. Urol.* **160,** 2381–2392.
8. Cunha, G. R., Donjacour, A. A., Cooke, P. S., Mee, S., Bigsby, R. M., Higgins, S. J. & Sugimura, Y. (1987) *Endocr. Rev.* **8,** 338–362.
9. Tsujimura, A., Koikawa, Y., Salm, S., Takao, T., Coetzee, S., Moscatelli, D., Shapiro, E., Lepor, H., Sun, T. T. & Wilson, E. L. (2002) *J. Cell Biol.* **157,** 1257–1265.
10. Spangrude, G. J., Heimfeld, S. & Weissman, I. L. (1988) *Science* **241,** 58–62.
11. Matsuura, K., Nagai, T., Nishigaki, N., Oyama, T., Nishi, J., Wada, H., Sano, M., Toko, H., Akazawa, H., Sato, T., *et al.* (2004) *J. Biol. Chem.* **279,** 11384–11391.
12. Welm, B. E., Tepera, S. B., Venezia, T., Graubert, T. A., Rosen, J. M. & Goodell, M. A. (2002) *Dev. Biol.* **245,** 42–56.
13. Montanaro, F., Liadaki, K., Volinski, J., Flint, A. & Kunkel, L. M. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 9336–9341.
14. Asakura, A. (2003) *Trends Cardiovasc. Med.* **13,** 123–128.
15. Falciatori, I., Borsellino, G., Haliassos, N., Boitani, C., Corallini, S., Battistini, L., Bernardi, G., Stefanini, M. & Vicini, E. (2004) *FASEB J.* **18,** 376–378.
16. Suzuki, A., Zheng, Y., Kondo, R., Kusakabe, M., Takada, Y., Fukao, K., Nakauchi, H. & Taniguchi, H. (2000) *Hepatology* **32,** 1230–1239.
17. Tani, H., Morris, R. J. & Kaur, P. (2000) *Proc. Natl. Acad. Sci. USA* **97,** 10960–10965.
18. Shinohara, T., Avarbock, M. R. & Brinster, R. L. (1999) *Proc. Natl. Acad. Sci. USA* **96,** 5504–5509.
19. Adams, J. M. & Cory, S. (1998) *Science* **281,** 1322–1326.
20. Domen, J., Cheshier, S. H. & Weissman, I. L. (2000) *J. Exp. Med.* **191,** 253–264.
21. Tiberio, R., Marconi, A., Fila, C., Fumelli, C., Pignatti, M., Krajewski, S., Giannetti, A., Reed, J. C. & Pincelli, C. (2002) *FEBS Lett.* **524,** 139–144.
22. Potten, C. S., Wilson, J. W. & Booth, C. (1997) *Stem Cells* **15,** 82–93.
23. Cunha, G. R. & Donjacour, A. (1987) *Prog. Clin. Biol. Res.* **239,** 273–282.
24. Norman, J. T., Cunha, G. R. & Sugimura, Y. (1986) *Prostate* **8,** 209–220.
25. Xin, L., Ide, H., Kim, Y., Dubey, P. & Witte, O. N. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 11896–11903.
26. Goodell, M. A., Brose, K., Paradis, G., Conner, A. S. & Mulligan, R. C. (1996) *J. Exp. Med.* **183,** 1797–1806.
27. Gussoni, E., Soneoka, Y., Strickland, C. D., Buzney, E. A., Khan, M. K., Flint, A. F., Kunkel, L. M. & Mulligan, R. C. (1999) *Nature* **401,** 390–394.
28. Bonyadi, M., Waldman, S. D., Liu, D., Aubin, J. E., Grynpas, M. D. & Stanford, W. L. (2003) *Proc. Natl. Acad. Sci. USA* **100,** 5840–5845.
29. Ito, C. Y., Li, C. Y., Bernstein, A., Dick, J. E. & Stanford, W. L. (2003) *Blood* **101,** 517–523.
30. Fortunel, N. O., Otu, H. H., Ng, H. H., Chen, J., Mu, X., Chevassut, T., Li, X., Joseph, M., Bailey, C., Hatzfeld, J. A., *et al.* (2003) *Science* **302,** 393.
31. Li, A., Simmons, P. J. & Kaur, P. (1998) *Proc. Natl. Acad. Sci. USA* **95,** 3902–3907.
32. Kaur, P. & Li, A. (2000) *J. Invest. Dermatol.* **114,** 413–420.
33. Watt, F. M. (2002) *EMBO J.* **21,** 3919–3926.
34. Jones, P. H. & Watt, F. M. (1993) *Cell* **73,** 713–724.
35. Bickenbach, J. R. & Chism, E. (1998) *Exp. Cell Res.* **244,** 184–195.
36. Collins, A. T., Habib, F. K., Maitland, N. J. & Neal, D. E. (2001) *J. Cell Sci.* **114,** 3865–3872.
37. Campos, L. S., Leone, D. P., Relvas, J. B., Brakebusch, C., Fassler, R., Suter, U. & ffrench-Constant, C. (2004) *Development (Cambridge, U.K.)* **131,** 3433–3444.
38. Fuchs, E., Tumbar, T. & Guasch, G. (2004) *Cell* **116,** 769–778.
39. Chung, J. & Mercurio, A. M. (2004) *Mol. Cells* **17,** 203–209.
40. Chao, C., Lotz, M. M., Clarke, A. C. & Mercurio, A. M. (1996) *Cancer Res.* **56,** 4811–4819.
41. Cress, A. E., Rabinovitz, I., Zhu, W. & Nagle, R. B. (1995) *Cancer Metastasis Rev.* **14,** 219–228.
42. Hockenbery, D. M., Zutter, M., Hickey, W., Nahm, M. & Korsmeyer, S. J. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 6961–6965.
43. Domen, J. & Weissman, I. L. (2000) *J. Exp. Med.* **192,** 1707–1718.
44. Domen, J. & Weissman, I. L. (2003) *Exp. Hematol.* **31,** 631–639.
45. Kyprianou, N. & Isaacs, J. T. (1989) *Mol. Endocrinol.* **3,** 1515–1522.
46. Rouleau, M., Leger, J. & Tenniswood, M. (1990) *Mol. Endocrinol.* **4,** 2003–2013.
47. Sugimura, Y., Cunha, G. R. & Donjacour, A. A. (1986) *Biol. Reprod.* **34,** 973–983.
48. Colombel, M., Vacherot, F., Diez, S. G., Fontaine, E., Buttyan, R. & Chopin, D. (1998) *Br. J. Urol.* **82,** 380–385.
49. De Marzo, A. M., Marchi, V. L., Epstein, J. I. & Nelson, W. G. (1999) *Am. J. Pathol.* **155,** 1985–1992.
50. McDonnell, T. J., Troncoso, P., Brisbay, S. M., Logothetis, C., Chung, L. W., Hsieh, J. T., Tu, S. M. & Campbell, M. L. (1992) *Cancer Res.* **52,** 6940–6944.
51. Bruckheimer, E. M., Spurgers, K., Weigel, N. L., Logothetis, C. & McDonnell, T. J. (2003) *J. Urol.* **169,** 1553–1557.
52. Richardson, G. D., Robson, C. N., Lang, S. H., Neal, D. E., Maitland, N. J. & Collins, A. T. (2004) *J. Cell Sci.* **117,** 3539–3545.
53. Molofsky, A. V., Pardal, R. & Morrison, S. J. (2004) *Curr. Opin. Cell Biol.* **16,** 700–707.
54. Karhadkar, S. S., Bova, G. S., Abdallah, N., Dhara, S., Gardner, D., Maitra, A., Isaacs, J. T., Berman, D. M. & Beachy, P. A. (2004) *Nature* **431,** 707–712.
55. Valk-Lingbeek, M. E., Bruggeman, S. W. & van Lohuizen, M. (2004) *Cell* **118,** 409–418.
56. Varambally, S., Dhanasekaran, S. M., Zhou, M., Barrette, T. R., Kumar-Sinha, C., Sanda, M. G., Ghosh, D., Pienta, K. J., Sewalt, R. G., Otte, A. P., *et al.* (2002) *Nature* **419,** 624–629.